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Soil functional diversity and P solubilization from rock phosphate after inoculation with native or allochtonous arbuscular mycorrhizal fungi

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Abstract

The potential benefits of inoculation with AM (arbuscular mycorrhizal) fungi were investigated on Atlas Cypress (*Cupressus atlantica* G.), an endemic Cupressacea in Morocco. The parameters under study were (i) the growth of the plant, (ii) the functional diversity of soil microflora and (iii) the rock phosphate (RP) solubilizing activity. *C. atlantica* growth was measured after 12 months of culture in plastic bags arranged in a randomised complete block design with 10 replicates per treatment. Fungal inoculation consisted of either *Glomus intraradices* alone or a mixture of native AM fungi. P amendment was supplied under the form of Khouribga Rock Phosphate (KRP) powder. Microbial catabolic diversity was assessed by measuring CO_2 production of SIR (substrate induced respiration) responses. Results showed that: (i) the fungal symbionts were effective to improve the growth of *C. atlantica*, confirming the requirement of mycorrhizal symbiosis for the successful establishment of *C. atlantica* in a degraded soil; (ii) *G. intraradices* appeared to be the most effective in promoting growth of *C. atlantica*, whereas indigenous AM fungi were relatively ineffective. Native AM fungi inoculation strongly modified functional abilities of the soil microflora, and in the treatments with P amendment, growth stimulations of native AM fungi inoculation were significantly higher than those of *G. intraradices* inoculation for the shoot growth and leaf P content; (iii) *C. atlantica* plants inoculated with native AM fungi could mobilize P from KRP more efficiently than those mycorrhized with *G. intraradices*. A strong interaction between KRP amendment and fungus inoculation was detected for the leaf P content results. In conclusion, the use of a mixture of native AM fungi combination may increase the chance of including one very effective fungal isolate, but also, creates a more favourable environment for the development of ecosystems processes.

Keywords: Arbuscular mycorrhizas; Diversity; Soil functional abilities; Rock phosphate; Morocco

1. Introduction

Mediterranean ecosystems are subjected to disturbances following scarce and irregular rainfall, long dry and hot summers and man-mediated degradative activities (overgrazing, non-regulated cultivation techniques, deforestation, etc.). Such degraded ecosystems are usually characterised by a disturbed vegetation cover accompanied by a rapid erosion of surface soil (Herrera et al., 1993). The desertification process involves a loss or reduction of major physicochemical and biological soil properties (Requena et al., 2001) and significantly reduced arbuscular mycorrhizal (AM) soil inoculum potential (Jasper et al., 1989, 1991; Brundrett, 1991; Herrera et al., 1993; Duponnois et al., 2001a). AM fungi

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have been found to be essential components of sustainable soil-plant systems (Smith and Read, 1997; van der Hejden et al., 1998; Schreiner et al., 2003) and particularly important in counteracting desertification of Mediterranean ecosystems (Carpenter and Allen, 1988; Brundrett, 1991). They increase plant uptake of phosphorus (Duponnois et al., 2005), micronutrients (Bürkert and Robson, 1994) and nitrogen (Barea et al., 1991). They enhance water absorption (George et al., 1992) and act as antagonists against some plant pathogens (Dehne, 1982; Lendzemo et al., 2005). AM fungal symbiosis changes root functions (i.e. root exudation) (Graham et al., 1981; Marshner et al., 1997), modifies carbohydrate metabolism of the host plant (Shachar-Hill et al., 1995) and interacts with rhizosphere populations (Hayman, 1983; Azaizeh et al., 1995; Andrade et al., 1997, 1998). The structure and functionalities of these AM associated microbial communities differ from those of the rhizosphere (Duponnois et al., 2005) and this microbial compartment has been named ''mycorrhizosphere'' (Linderman, 1988).

Plant mineral nutrition depends mainly on the phosphorus content of soil, which can be assimilated only as soluble phosphate. Hence the use of rock phosphate (RP) as a fertilizer for P-deficient soils has received significant interest in recent years since they are natural, inexpensive and available fertilizers. However their solubilization rarely occurs in non-acidic soils (Caravaca et al., 2004, 2005a). Physical and chemical weathering of mineral phosphates is mainly realised along plant roots in the rhizosphere. This part of soil supports large microbial communities that facilitate weathering of minerals by producing organic acids, phenolic compounds, protons and siderophores (Drever and Vance, 1994; Landeweert et al., 2001). Among microbial groups that could solubilize mineral phosphates and improve plant phosphorus nutrition are AM fungi. Arbuscular mycorrhizal fungal inoculation induced spectacular stimulations of the plant growth and P foliar content (Guissou et al., 2001; Caravaca et al., 2004, 2005a,b; Duponnois et al., 2005).

It is well known that AM fungal inoculum potential is very low in degraded Mediterranean ecosystems and an increase of this fungal inoculum potential is needed in both natural and artificial revegetation processes (McGee, 1989). It has already been shown that AM inoculation of plants is very efficient in establishing plants on disturbed soils (Estaun et al., 1997; Duponnois et al., 2001b). There are two main approaches in order to increase and maintain high populations of infective AM propagules in soil: (i) screening of AM fungal isolates (native or exotic isolates) for their effect on the plant growth under controlled conditions and an inoculation of the soil with the most efficient AM strains and (ii) adoption of field practices to manage and improve the inoculum potential of indigenous mycorrhizae. In forestry, the inoculation practice is generally used in tree nurseries in order to help tree establishment (Plenchette, 2000), but also to improve the quality of the planted soil (Franson and Bethlenfalvay, 1989). In a previous study, controlled mycorrhization with an allochtonous AM fungus (*Glomus intraradices*) has significantly improved the growth of *Cupressus atlantica* and strongly modified soil microbial functionalities (Ouahmane et al., 2006). Although it has been shown that native AM fungi are important contributors to ecosystem productivity and functioning (van der Hejden et al., 1998; Requena et al., 2001; Alguacil et al., 2005; Caravaca et al., 2005b), little is known about the potential impact of native AM fungi on the host plant mycorrhizal dependency, microbial soil biofunctioning and subsequent biological processes (i.e. RP weathering).

The main objectives of this investigation were to assess how inoculation with a mixture of native AM fungi or a single AM inoculation with *G. intraradices* affect (i) the growth of Atlas Cypress (*C. atlantica* G.), an endemic Cupressacea of Morocco, (ii) the functional diversity of soil microflora and to determine (iii) if microbial activity increased P uptake from RP under the tested conditions.

2. Materials and methods

2.1. Preparation of fungal inoculums

Soil samples were collected from the rhizosphere of *C. atlantica* at the Idni station (8°17′02″W, 31°54′34″, 1700 m above sea level) located in the N'Fis valley (Haut Atlas, Morocco). They were taken from 10 individual trees, 2 m from the trunk under the canopy. Each sample consisted of five 100 g sub-samples collected at the 20 cm depth. The soil was carefully mixed and spores of AM fungi were extracted from each sub-sample (100 g) by wet sieving and decanting, followed by sucrose centrifugation (Sieverding, 1991). The supernatant was poured through a 50 μm sieve and rinsed with tap water. Fungal spores were surface sterilized with a solution of chloramine T (0.2 g l^{-1}) and streptomycine (0.2 g l^{-1}) (Mosse, 1973) in order to eliminate the mycorrhizosphere microflora. Then they were kept in distilled water at 4 °C for 2 days before use.

The AM fungus *G. intraradices* Schenk & Smith (DAOM 181602, Ottawa Agricultural Herbarium) was multiplied on leek (*Allium porrum* L.) on Terragreen™ substrate. This culture substrate (calcined clay, average particle size 5 mm) used for propagation of AM fungi is an attapulgite from Georgia (Plenchette et al., 1996). After 12 weeks under greenhouse conditions, leek plants were uprooted, gently washed and roots cut into 0.5 cm long pieces bearing around 250 vesicles cm^{-1}.

Seeds of maize (*Zea maize* L.) were surface-sterilized with 1% NaOCl for 15 min and rinsed with distilled water. They were pre-germinated for 2 days in Petri dishes on humid filter paper at 25 °C in the dark. The germinating seeds were used when rootlets were 1–2 cm long.

The soil was collected under *C. atlantica* in High Atlas Mountains (Morocco), crushed, passed through a 2 mm sieve and autoclaved (140 °C, 40 min) to eliminate native micro-organisms. After autoclaving, its chemical characteristics were as follows: pH (H_2O) 7.7; clay (%) 4.4; fine silt (%) 28.6; coarse silt (%) 12.6; fine sand (%) 27.9; coarse sand (%) 19.45; carbon (%) 3.15; nitrogen (%) 0.14; C/N 23; P (Olsen) 13.1 mg kg^{-1}. Black plastic bags (1 dm^3) were filled with this autoclaved soil.

A hole (1 cm × 5 cm) was made in the soil of each plastic bag and filled (i) with 2 g (fresh weight) leek root pieces infected with *G. intraradices* or (ii) with water suspensions of native AM fungal spore mixture collected from the rhizosphere of *C. atlantica* at the Idni station or (iii) with 2 g (fresh weight) non-mycorrhizal leek root pieces. Holes were covered with the same autoclaved soil and one pre-germinated maize seed was planted per plastic bag. There were 10 replicates per treatment. The plastic bags were placed in a greenhouse under natural light (daylight approximately 12 h, daily average temperature 25 °C).

After 6 months of culturing, the maize plants were uprooted and their root systems were gently washed. One root sub-sample (0.5 g fresh weight) was collected from each plastic bag and their internal colonization by AM fungi was quantified. Roots were cleared and stained according to the method of Phillips and Hayman (1970). The root pieces were placed on a slide for microscopic observation under 250× magnification (Brundrett et al., 1985). About fifty 1 cm root pieces were randomly chosen from each root sub-sample and observed per plant. Extent of mycorrhizal colonization was expressed in terms of fraction of root length with mycorrhizal internal structures (vesicles or hyphae): (length of root fragments colonized/total length of root fragments) × 100. In addition, spores of AM fungi were extracted from each sub-sample (100 g per plastic bag) as described above. Spores were identified mainly using spore size and color, wall structure and hyphal attachment. Root systems of maize plants from each treatment (inoculated or not) were mixed, cut into 1 cm root pieces and kept at 4 °C for further use.

2.2. Glasshouse experiment

Seeds of *C. atlantica* (Provenance Idni, Morocco) were surface sterilized with 30% hydrogen peroxide for 5 min, rinsed and soaked for 24 h in 4 °C sterile distilled water. Seeds were then transferred to Petri dishes (100 seeds per dish) on paper soaked in sterile distilled water. Plates were then incubated at 20 °C. The germinating seeds were used when rootlets were 1–2 cm long.

The same sterilized soil as before was used. It was mixed with Khouribga Rock Phosphate (KRP) (0.1%, w/v; insoluble rock phosphate powder) in one treatment or remained unamended in another. Khouribga Rock Phosphate (Morocco) was ground with pestle and mortar and passed through a 90 μm sieve. Its chemical characteristics were as follows (%): SiO_2 3.1; Al_2O_3 0.5; Fe_2O_3 0.27; P_2O_5 33.4; MgO 0.5; CaO 54; K_2O 0.06; Na_2O 0.76; CaP 2.64; CO_2 2.1 (Hafidi, 1996).

Each plastic bag (2 dm^3) was filled with the soil mixture and planted with one pre-germinated seed of *C. atlantica*. Arbuscular mycorrhizal inoculation was done as described before using fungal inoculums propagated on maize plants. Non-mycorrhizal *Zea mays* roots, prepared as above, were used for the control treatment. One hole was made in each plastic bag and filled with 1 g fresh disinfected maize roots (*G. intraradices* mycorrhizal roots, roots mycorrhized with a mixture of native AM fungi; or non-mycorrhizal roots). The holes were then covered with the same sterilized soil but newly sampled. Plants were daily watered with tap water (pH 6.0). The plastic bags were arranged in a randomised complete block design with 10 replicates per treatment. They were placed in a glasshouse under natural light (daylight approximately 12 h, daily average temperature 18 °C).

After 12 months of culturing, *C. atlantica* seedlings were uprooted and their root systems were gently washed. Height and dry weight of the shoots (1 week at 65 °C) were measured. Mycorrhizal dependency of *C. atlantica* was determined by expressing the difference between the total dry weight of the mycorrhizal plant and the total dry weight of the non-mycorrhizal plant, as a percentage of the total dry weight of the mycorrhizal plant (Plenchette et al., 1983). After drying plant tissues were ground, ashed (500 °C), digested in 2 ml HCL 6N and 10 ml HNO_3 N and then analysed by colorimetry for P (John, 1970). The internal colonization of AM fungi along the root systems was quantified as describe above. Then the soil of each plastic bag was mixed and AM spores were extracted as described above.

2.3. Measurement of microbial catabolic diversity

Microbial catabolic diversity was assessed by adding a range of 28 simple organic compounds to the soil and determining the short-term respiration responses (Degens and Harris, 1997). Measurements were done on three soil samples cored from bags randomly chosen from each treatment. They were collected along *C. atlantica* root systems and could be interpreted as a mixture of ectomycorrhizosphere and hyphosphere soils. Each of the 28 substrates suspended in 2 ml sterile distilled water was added to 1 g of dry soil in 10 ml bottles (West and Sparling, 1986). CO_2 production from basal respiratory activity in the soil samples was measured by adding 2 ml sterile distilled water to 1 g equivalent dry weight of soil. After the addition of the substrate solutions to soil samples, bottles were immediately sealed with a Vacutainer stopper and incubated at 28 °C for 4 h in darkness. After 4 h, respired CO_2 in the headspace of each bottle was determined by taking a 1 ml syringe sample and by analysing CO_2 concentration using an infrared gas analyser (IRGA) (Polytron IR CO_2, Dräger^{TM}) in combination with a thermal flow meter (Heinemeyer et al., 1989). Results were subtracted from the CO_2 basal production expressed as $\mu g\ CO_2\ g^{-1}$ soil h^{-1}. Among 28 substrates, there were eight amino acids (L-glutamine, L-serine, L-arginine, L-asparagine, L-histidine, L-lysine, L-glutamic acid, L-tyrosine), three carbohydrates (D-glucose, D-mannose, sucrose), three amides (D-glucosamine, *N*-methyl-D-glucamine, succinamide) and 14 carboxylic acids (ascorbic acid, citric acid, fumaric acid, gluconic acid, quinic acid, malonic acid, α-ketoglutaric acid, α-ketobutyric acid, succinic acid, tartaric acid, uric acid, oxalic acid, malic acid, hydroxybutyric acid). The amines and amino acids were added at 10 mM whereas the carbohydrates were added at 75 mM and the carboxylic acids at 100 mM (Degens and Vojvodic-Vukovic, 1999). Catabolic richness and catabolic evenness were calculated to evaluate the catabolic diversity of soil treatments. Catabolic richness, *R*, expressed the number of

substrates used by microorganisms in each soil treatment. Catabolic evenness, E, representing the variability of a substrate among the range of tested substrates, was calculated using the Simpson–Yule index, $E = 1/p_i^2$ with p_i = respiration response to individual substrates/total respiration activity induced by all substrates for a soil treatment (Magurran, 1988). The catabolic evenness is a component of microbial functional diversity and is commonly defined as the uniformity of substrate use by soil microflora.

2.4. Statistical analysis

Data were treated with one-way and two-way analysis of variance. Means were compared using the Newman & Keuls test ($p < 0.05$). Two-way analysis of variance was used to test the interaction between the type of inoculation (control, *G. intraradices* or mixture of native AM fungi) and rock phosphate amendment (presence or absence).

The percentages of mycorrhization were arcsin ($\sqrt{x}$) transformed before statistical analysis.

Co-inertia analysis (COIA, Dray et al., 2003) was used to analyse the relationships between the plant growth parameters and the SIR responses. COIA is a multivariate analysis technique that describes the relationships between two data tables by searching successive pairs of axes with maximum covariance (instead of correlation, as in canonical correlation analysis). Monte Carlo (or permutation) tests were used to check the significance of the relationships between the two tables. Computations and graphical displays were made with the free ADE-4 software (Thioulouse et al., 1997), which is available on Internet at http://pbil.univ-lyon1.fr/ADE-4/.

3. Results

The percentages of maize root colonization by *G. intraradices* and by mixture of native AM fungi were $72.4 \pm 5.3\%$ and $57.3 \pm 6.4\%$ (C25), respectively. Native AM fungal inoculum consisted of a mixture of three AMF taxa (*Scutellospora* spp., *Glomus* spp. and *Acaulospora* spp.). After 12 months of culturing, these three genera were detected in the native AM fungi inoculated soil treatments. The not inoculated treatment (control) remained not colonized at the end of the experiment.

The Monte Carlo test of the co-inertia analysis between the plant growth parameters and the SIR responses was highly significant. Fig. 1 shows the results of this analysis. Fig. 1A and B shows the variable loadings, for the plant growth parameters (Fig. 1A), and for the SIR responses (Fig. 1B). Fig. 1C and D shows the soil sample scores, for the plant growth parameters table (Fig. 1C) and for the SIR responses table (Fig. 1D). It is important to note that these four graphics are at the same scale and could be superimposed.

The plant growth parameter graphic (Fig. 1A) shows a ''size effect'' with better growth toward the left, and a ''shape effect'' with longer shoots downward. The SIR substrates graphic (Fig. 1B) shows that the substrates consumed in high plant growth conditions (left of the graphic) are uric, citric and ketobutyric acids, together with mannose, serine, histidine and lysine. Conversely, substrates metabolized in low plant growth conditions (left of the graphic) are cyclohexane and glutamine.

The effect of inoculations and of P amendment on SIR responses can be seen in Fig. 1D. On this figure, a shift to the left corresponds to a higher use of uric, citric and ketobutyric acids, and to a lower use of cyclohexane and glutamine. The effect of native AM fungal inoculation (solid arrow) is stronger than the effect of *G. intraradices* inoculation (dash-dot arrow). The effect of P amendment on the control (dashed arrow) is comparable to the effect of *G. intraradices* inoculation (dash-dot arrow). The effect of P amendment (dashed arrows) is the same on native AM fungal inoculation inoculated samples and on *G. intraradices* inoculated samples.

The effects of inoculations and of P amendment on plant growth can be seen in Fig. 1C. On this figure, a shift to the left corresponds to better plant growth. There was no effect of phosphorus amendment on plant growth for control samples and for *G. intraradices* inoculated samples. Native AM fungal inoculation and *G. intraradices* inoculation both had positive effects on plant growth compared to the control, but phosphorus amendment is much more effective on native arbuscular mycorrhizal fungi inoculated samples.

The leaf P content was positively influenced by the interaction between KRP amendment and fungus inoculation (two-way analysis of variance: $p < 10^{-4}$), while shoot height, biomasses and mycorrhization were not influenced.

In the treatments without P amendment, fungal inoculations significantly improved plant growth and leaf P content, except for the height of *G. intraradices* inoculated plants, which was not significantly different from the control (Table 1). The *G. intraradices* effect was significantly higher than that of native AM fungi inoculation for the root biomass and for the root/shoot ratio (Table 1).

In the treatments with P amendment, the positive influence of AM inoculation was recorded on the plant growth except for the height of plants inoculated with *G. intraradices* where no significant fungal effect was recorded compared to the control (Table 2). Growth stimulations of native AM fungi inoculation were significantly higher than those measured in the *G. intraradices* treatment for the shoot growth and leaf P content (Table 2). Root/shoot ratio was significantly higher in the AM fungal treatments than in the control (Table 2).

The *G. intraradices* effect on plant growth was not affected by KRP amendment as no significant differences were recorded between the development of *G. intraradices* inoculated plants in the soils with or without KRP amendments (Table 3). On the contrary, the native AM fungi effect on plant growth was significantly higher in the soil amended with KRP than in the non-amended soil (Table 3). For the control treatment, KRP amendment has significantly decreased leaf P content (Table 3).

In the non-amended soils, the catabolic richness was significantly lower in the *G. intraradices* inoculated soil than in the control, whereas its catabolic evenness was significantly higher than in the non-inoculated soil (Table 4). Compared to the control, average SIR responses with carboxylic acids, amides and carbohydrates were significantly higher in the native AM fungi inoculated soil (Table 4).

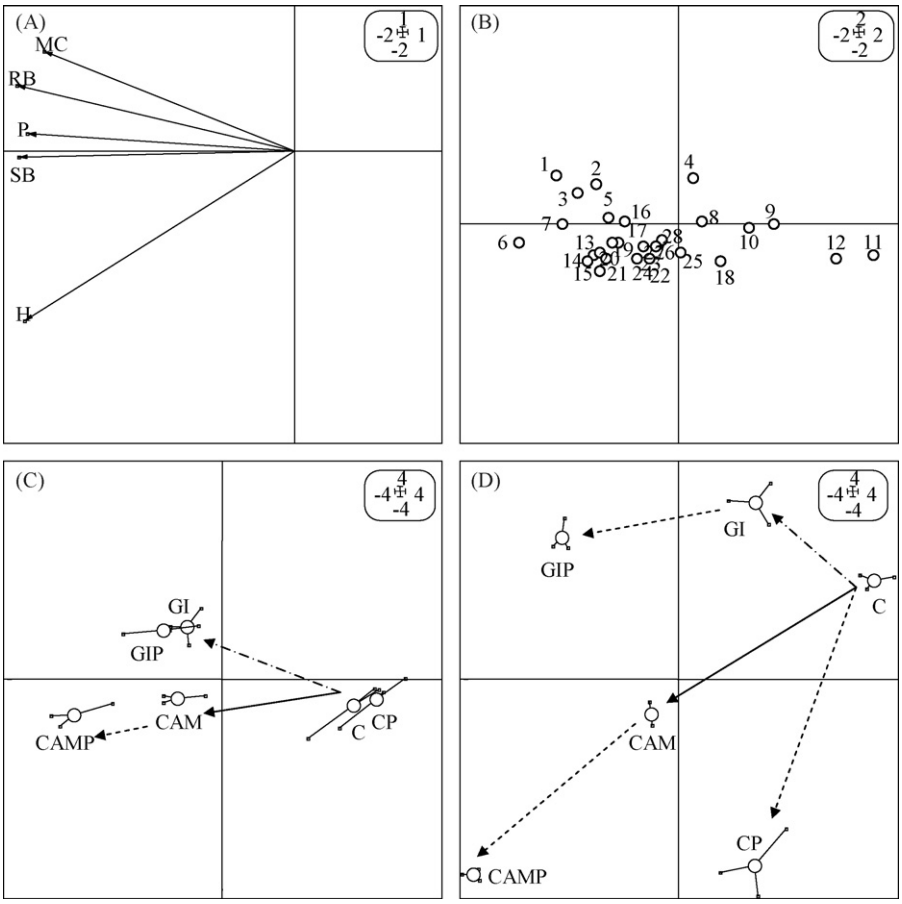

Fig. 1. Co-inertia analysis of the SIR responses of the soils inoculated with *G. intraradices* or the mixture of native arbuscular mycorrhizal fungi and/or Khouribga Rock Phosphate, plant growth, phosphorus leaf content and mycorrhizal colonization. (A) Factor map of plant growth and mycorrhizal colonization variables (H, height; SB, shoot biomass; RB, root biomass; P, leaf P content; MC, mycorrhizal colonization). (B) Factor map of SIR responses (D-mannose, 1; L-serine, 2; L-histidine, 3; L-tyrosine, 4; gluconic acid, 5; uric acid, 6; L-lysine, 7; L-glutamic acid, 8; sucrose, 9; succinamide, 10; cyclohexane, 11; L-glutamine, 12; citric acid, 13; α-ketobutyric acid, 14; tartaric acid, 15; DL-α-hydroxybutyric acid, 16; *N*-methyl-D-glucosamine, 17; D-glucose, 18; quinic acid, 19; L-asparagine, 20; succinic acid, 21; malic acid, 22; oxalic acid, 23; fumaric acid, 24; ascorbic acid, 25; malonic acid, 26; α-ketoglutaric acid, 27; L-arginine, 28). (C) Factor map of plant growth and mycorrhizal colonization (C, control (not inoculated); CP, Khouribga Rock Phosphate amendment; GI, *G. intraradices* inoculation; GIP, *G. intraradices* inoculation and Khouribga Rock Phosphate amendment; CAM, mixture of native arbuscular mycorrhizal fungi inoculation; CAMP, CAM inoculation and Khouribga Rock Phosphate amendment). (D) Factor map of SIR responses soil samples (for the legend, see (C)).

In the RP amended soil, no significant differences were recorded for the catabolic richness or average SIR responses with amides and carbohydrates (Table 4). The catabolic evenness was significantly higher in the *G. intraradices* inoculated soil than in the other treatments and the highest average SIR response with carboxylic acids was found in the native AM fungi inoculated soil (Table 4).

Table 1
Growth response of *C. atlantica*, leaf phosphorus content and mycorrhizal colonization in soils inoculated with *G. intraradices* or with the mixture of native AM fungi after 12-month culture in a disinfected soil

	Treatments		
	Control	*G. intraradices*	Native AM fungi
Height (cm)	21.3 (1.33)[a] a[b]	21.8 (0.73) a	25.7 (0.33) b
Shoot biomass (g dry weight)	1.86 (0.12) a	3.06 (0.07) b	3.12 (0.11) b
Root biomass (g dry weight)	0.63 (0.04) a	1.41 (0.07) c	1.03 (0.06) b
Total biomass (g dry weight)	2.49 (0.16) a	4.46 (0.12) b	4.15 (0.14) b
Root/shoot ratio	0.34 (0.002) a	0.46 (0.02) b	0.33 (0.016) a
Mycorrhizal dependency (%)		44.1 (1.59) a	39.9 (2.1) a
Leaf P content (mg per plant)	0.51 (0.032) a	0.65 (0.022) b	0.65 (0.023) b
Mycorrhizal colonization (%)	0 a	42.6 b	39.4 b

[a] S.E. of the mean.
[b] Data in the same line followed by the same letter are not significantly different according to the Newman & Keuls test ($p < 0.05$).

Table 2
Effect of *G. intraradices* or native AM fungi inoculation on the growth, leaf phosphorus content and mycorrhizal colonization of *C. atlantica* in a disinfected soil amended with Khouribga Rock Phosphate (KRP) after 12-month culture

	Treatments		
	Control	*G. intraradices*	Native AM fungi
Height (cm)	20.7 (1.2)[a] a[b]	23.0 (0.6) a	29.0 (1.0) b
Shoot biomass (g dry weight)	1.95 (0.11) a	2.90 (0.17) b	3.43 (0.02) c
Root biomass (g dry weight)	0.67 (0.05) a	1.71 (0.15) b	1.82 (0.08) b
Total biomass (g dry weight)	2.62 (0.15) a	4.61 (0.32) b	5.25 (0.06) b
Root/shoot ratio	0.35 (0.018) a	0.59 (0.02) b	0.53 (0.03) b
Mycorrhizal dependency (%)		42.6 (4.01) a	50.1 (0.56) a
Leaf P content (mg per plant)	0.38 (0.021) a	0.66 (0.02) b	0.82 (0.005) c
Mycorrhizal colonization (%)	0 a	43.2 b	40.3 b

[a] S.E. of the mean.
[b] Data in the same line followed by the same letter are not significantly different according to the Newman & Keuls test ($p < 0.05$).

Table 3
Comparison between *G. intraradices* or native AM fungi inoculation impact on growth and leaf phosphorus content of *C. atlantica* seedlings in both soil treatments with or without Khouribga Rock Phosphate (KRP) amendment after 12-month culture

	Treatments		
	Control	*G. intraradices*	Native AM fungi
Height (cm)	NS[a]	NS	*($p = 0.034$) (+11.5)
Shoot biomass (g dry weight)	NS	NS	*($p = 0.042$) (+9.1)
Root biomass (g dry weight)	NS	NS	*($p = 0.036$) (+38.3)
Total biomass (g dry weight)	NS	NS	*($p = 0.016$) (+18.7)
Leaf P content (mg per plant)	*[b]($p = 0.024$) (−35.9)[c]	NS	*($p = 0.002$) (+21.6)

[a] Not significant.
[b] *Significantly different according to the Student "*t*" test ($p < 0.05$).
[c] Percentage of stimulation or inhibition.

The highest SIR responses with carboxylic acids known to be involved in the process of P solubilization were recorded from the native AM fungi treatment in the non-amended soil (Table 5). In the KRP amended soil, SIR responses with citric

Table 4
Catabolic richness, catabolic evenness and average substrate-induced respiration (SIR) responses ($\mu g\ CO_2\ g^{-1}$ soil h^{-1}) with each substrate group (carboxylic acids, amino-acids, amides and carbohydrates) in the soil treatments

	Treatments		
	Control	*G. intraradices*	Native AM fungi
Without KRP[a] amendment			
Catabolic richness	28.0 b[b]	26.7 a	27.7 ab
Catabolic evenness	12.0 b	12.2 c	11.7 a
Carboxylic acids	2595.5 a	3224.8 b	4214.3 c
Amino-acids	100.4 ab	88.8 a	106.7 b
Amides	50.0 a	50.0 a	84.4 b
Carbohydrates	94.4 a	110.0 ab	122.2 b
With KRP amendment			
Catabolic richness	28.0 a	28.0 a	28.0 a
Catabolic evenness	11.8 a	12.7 b	11.8 a
Carboxylic acids	5268.3 b	3683.8 a	4214.3 c
Amino-acids	129.6 a	143.3 b	145.8 b
Amides	90.0 a	85.6 a	84.4 a
Carbohydrates	202.2 a	176.7 a	167.8 a

[a] Khouribga Rock Phosphate.
[b] Data in the same line followed by the same letter are not significantly different according to the Newman & Keuls test ($p < 0.05$).

Table 5
Substrate-induced respiration (SIR) responses ($\mu g\ CO_2\ g^{-1}$ soil h^{-1}) with carboxylic acids known to be involved in the process of phosphate solubilization

	Treatments		
	Control	*G. intraradices*	Native AM fungi
Without KRP[a] amendment			
Citric acid	1520 (26.4)[b] a[c]	2457 (26.0) b	3830 (20.8) c
Malonic acid	1347 (18.6) a	4067 (35.3) b	4550 (70.1) c
Fumaric acid	2993 (100.4) a	3910 (55.1) b	5620 (83.9) c
Malic acid	3947 (76.2) a	4303 (108.9) a	6007 (75.3) b
Oxalic acid	4080 (68.1) a	4873.3 (40.9) b	6650 (79.4) c
Tartaric acid	4957 (61.7) b	4187 (40.6) a	6780 (83.9) c
Gluconic acid	1720 (32.1) a	1896 (3.3) a	2223 (8.8) b
With KRP amendment			
Citric acid	5633 (92.1) b	3683 (23.3) a	7417 (21.8) c
Malonic acid	7390 (80.8) c	3090 (34.6) a	6370 (37.8) b
Fumaric acid	7893 (93.3) b	4783 (49.1) a	7846 (93.8) b
Malic acid	8223 (76.2) b	5360 (37.9) a	8177 (112.9) b
Oxalic acid	7937 (63.8) b	5346 (42.6) a	7880 (127.1) b
Tartaric acid	6687 (52.4) b	5443 (68.9) b	8420 (80.8) c
Gluconic acid	2346 (23.3) b	2470 (35.1) c	2270 (11.6) a

[a] Khouribga Rock Phosphate.
[b] S.E. of the mean.
[c] Data in the same line followed by the same letter are not significantly different according to the Newman & Keuls test ($p < 0.05$).

and tartaric acids were higher in the native AM fungi treatment than in the control, whereas SIR responses with malonic acid and gluconic acid were larger in the control and in the *G. intraradices* treatment than in the other treatments (Table 5).

4. Discussion

From the results of this study, the following three main points deserve discussion: (i) whether the fungal symbionts were effective in improving the growth of *C. atlantica*, (ii) whether functional abilities of soil microflora depended on inoculated AM fungi and (iii) whether rock phosphate solubilization depended on inoculated AM fungi:

(i) The AM fungal genera identified in the mixture of native AM fungi (*Glomus*, *Scutellospora* and *Acaulospora*) are generally found in Mediterranean ecosystems (Requena et al., 2001). Some studies report AM colonization of *Cupressus* sp. (Michelsen et al., 1993) and our results demonstrate a high mycorrhizal dependency which confirms that *C. atlantica* could be considered as "obligatory mycorrhizal" (Brundrett, 1991) or as "highly dependent on mycorrhiza" (Habte and Manjunath, 1991; Ouahmane et al., 2006). These results demonstrate beneficial effects of mycorrhizal symbiosis for the successful growth of *C. atlantica* suggesting its importance for better establishment in degraded soil.

(ii) Assuming that a high root/shoot ratio reflects a high degree of AM effectiveness (Tobar et al., 1994), *G. intraradices* appeared to be the most effective in promoting growth of *C. atlantica*, whereas indigenous AM fungi were relatively ineffective. This may be due to the faster ability of this exotic fungal isolate to colonize plant roots making it highly competitive (Duponnois, unpublished data). This result is in accordance with those recorded from previous studies with other host plants such as *Anthyllis cytisoides* (Requena et al., 1996) and *Acacia holosericea* (Duponnois et al., 2005).

Conversely, native AM fungi inoculation strongly modified functional abilities of the soil microflora and, more specifically its capacity to metabolize carboxylic acids. It is well known that root mycorrhizal colonization changes the number of aerobic bacteria in the rhizosphere (Posta et al., 1994) and affects bacterial growth rate (Marshner and Crowley, 1996). Mycorrhizal roots also differ in microbial community structure of the rhizosphere from non-mycorrhizal roots (Fillion et al., 1999; Vazquez et al., 2000). This microbial compartment is commonly named "mycorrhizosphere" (Linderman, 1988). Moreover, it has been found that mycorrhizal fungi differ in their effect on the bacterial community composition in the rhizosphere (Timonen et al., 1998; Marshner and Baumann, 2003). This is confirmed in the present study through the measurement of microbial functional activities. The soil around the external mycelium constitutes another specific microbial compartment, commonly named "hyphosphere" which is influenced by AM fungal exudations and supports different bacterial activities from those recorded in the mycorrhizosphere (Andrade et al., 1998; Mansfeld-Giese et al., 2002; Marshner and Timonen, 2005). Microorganisms in the hyphosphere of AM fungi may affect mycorrhizal functions such as nutrient and water uptake carried out by the external hyphae of AM fungi. In the present study, the average SIR response with carboxylic acids was higher in the soil inoculated with native AM fungi. In the process of phosphate solubilization, among the identified carboxylic acids, dicarboxylic (oxalic, tartaric, malic, fumaric, malonic acids) and tricarboxylic acids (citric acid) were effective in phosphorus mobilization (Ryan et al., 2001). It has also been stated that gluconic acid was the most frequent agent for mineral phosphate solubilization (Illmer and Schinner, 1992). SIR responses with all these organic acids have been measured and the highest values were recorded in the native AM fungi treatment. It suggests that these AM fungi and their associated hyphosphere microflora excreted higher amounts of such organic acids. These organic compounds could exert a selective influence on soil microbial communities through a multiplication of microorganisms that catabolize organic acids.

(iii) *C. atlantica* plants inoculated with native AM fungi could mobilize P from KRP more efficiently than those mycorrhized with *G. intraradices*. Opposite results have been recorded in a previous study where *G. intraradices* inoculated to *A. holosericea* was found to be more efficient in mobilizing P from a Rock phosphate from Burkina Faso (Kodjari Rock Phosphate) than native AM fungi (Duponnois et al., 2005). This result suggests that AM fungal species had different abilities to weather rock phosphate and that some of the native AM fungi are very efficient to mobilize soluble P from the mineral phosphate.

It has been recently demonstrated that ectomycorrhizal symbiosis has an indirect positive effect on plant growth through its selective pressure on bacterial communities in addition to its well-known direct positive effect (Frey-Klett et al., 2005). In the present study, it has been demonstrated that AM fungi influenced functional characteristics of soil microbial communities and selected microorganisms potentially beneficial to the plant growth *via* their potential involvement in RP weathering. These microorganisms are also present in the non-inoculated soil (control) (high SIR responses with carboxylic acids) but these systems did not induce a better rock phosphate exploitation by the host plant. Hence, we conclude that the presence of AM fungi is obligatory in order to mobilize P from RP to the host plant. It is well known that AM symbiosis increases the explored volume of soil than roots alone and provide a greater surface area for phosphate uptake (Jakobsen et al., 1992). This result suggests that functional roles of AM symbiosis could result from a multitrophic microbial association, including hyphosphere and mycorrhizosphere communities (selected by AM symbiosis and plastic bagentially beneficial to the plant growth) and the fungal symbiosis.

In conclusion, it appears that, although classical controlled mycorrhization significantly improved plant growth, its impact on soil microflora was sometime inadequate to optimize the exploitation of the resources available in the system. The use of a native AM fungi mixture might increase the chance of including one very effective fungal isolate, but also create a more favourable environment for the development of ecosystem processes. These results correlate those of Caravaca et al. (2003) who concluded that the use of native mycorrhizal fungi as a source of AM inoculum might be a better strategy to improve plant growth in a degraded soil.

These conclusions do not question the utility of mycorrhizal inoculation in nursery practices. As the low density of mycorrhizal propagules may limit the successful plant re-establishment in degraded soils, it is necessary to increase indigenous inoculum levels of AM fungi in these ecosystems by appropriate mycorrhizal inoculation technologies (Caravaca et al., 2003). However, the use of native mycorrhizal fungi and their selective effects on soil microflora have to be considered in order to optimize the sustainable re-establishment of plant species in a degraded soil.

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References

Alguacil, M.M., Caravaca, F., Roldan, A., 2005. Changes in rhizosphere microbial activity mediated by native or allochtonous AM fungi in the reafforestation of a Mediterranean degraded environment. Biol. Fertil. Soils 41, 59–68.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1997. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant Soil 192, 71–79.

Andrade, G., Mihara, K.L., Linderman, R.G., Bethlenfalvay, G.J., 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere. Plant Soil 202, 89–96.

Azaizeh, H.A., Marshner, A., Römheld, V., Wittenmayer, L., 1995. Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exsudation of soil-grown maize plants. Mycorrhiza 5, 321–327.

Barea, J.M., El-Atrach, F., Azcon, R., 1991. The role of VA mycorrhizas in improving plant N acquisition from soil as assessed with ^{15}N. In: Fitton, C. (Ed.), The Use of Stable Isotopes in Plant Nutrition. Soil Fertility and Environmental Studies, Joint IAEA, FAO Division, Vienna, pp. 677–808.

Brundrett, M.C., 1991. Mycorrhizas in natural ecosystems. In: Macfayden, A., Begon, M., Fitter, A.H. (Eds.), Advances in Ecological Research, vol. 21. Academic Press Ltd., London, pp. 171–313.

Brundrett, M.C., Piche, Y., Peterson, R.L., 1985. A developmental study of the early stages in vesicular-arbuscular mycorrhizal formation. Can. J. Bot. 63, 184–194.

Bürkert, B., Robson, A., 1994. ^{65}Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root free sandy soil. Soil Biol. Biochem. 26, 1117–1124.

Caravaca, F., Barea, J.M., Palenzuela, J., Figueroa, D., Alguacil, M.M., Roldan, A., 2003. Establishment of shrub species in a degraded semiarid site after inoculation with native or allochtonous arbuscular mycorrhizal fungi. Appl. Soil Ecol. 22, 103–111.

Caravaca, F., Alguacil, M.M., Azcon, R., Diaz, G., Roldan, A., 2004. Comparing the effectiveness of mycorrhizal inoculum and amendment with sugar beet, rock phosphate and *Aspergillus niger* to enhance field performance of the leguminous shrub *Dorycnium pentaphyllum* L. Appl. Soil Ecol. 25, 169–180.

Caravaca, F., Alguacil, M.M., Azcon, R., Parladé, J., Torres, P., Roldan, A., 2005a. Establishment of two ectomycorrhizal shrub species in a semiarid site after *in situ* amendment with sugar beet, rock phosphate and *Aspergillus niger*. Microb. Ecol. 49, 73–82.

Caravaca, F., Alguacil, M.M., Torres, P., Roldan, A., 2005b. Survival of inocula and native AM fungi species associated with shrubs in a degraded Mediterranean ecosystem. Soil Biol. Biochem. 37, 227–233.

Carpenter, A.T., Allen, M.F., 1988. Responses of *Hedysarum boreale* Nutt. to mycorrhizas and Rhizobium: plant and soil nutrient changes in a disturbed shrub-steppe. New Phytol. 109, 125–132.

Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring the metabolic diversity of soil microbial communities. Soil Biol. Biochem. 29, 1309–1320.

Degens, B.P., Vojvodic-Vukovic, M., 1999. A sampling strategy to assess the effects of land use on microbial functional diversity in soils. Aust. J. Soil Res. 37, 593–601.

Dehne, H.W., 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72, 1115–1119.

Dray, S., Chessel, D., Thioulouse, J., 2003. Co-inertia analysis and the linking of ecological tables. Ecology 84, 3078–3089.

Drever, J.I., Vance, G.F., 1994. In: Lewan, M.D., Pittman, E.D. (Eds.), Role of Soil Organic Acids in Mineral Weathering Processes. The Role of Organic Acids in Geological Processes. Springer-Verlag, pp. 138–161.

Duponnois, R., Plenchette, C., Thioulouse, J., Cadet, P., 2001a. The mycorrhizal soil infectivity and arbuscular mycorrhizal fungal spore communities in soils of different aged fallows in Senegal. Appl. Soil Ecol. 17, 239–251.

Duponnois, R., Plenchette, C., Bâ, A.M., 2001b. Growth stimulation of seventeen fallow leguminous plants inoculated with *G. aggregatum* in Senegal. Eur. J. Soil Biol. 37, 181–186.

Duponnois, R., Colombet, A., Hien, V., Thioulouse, J., 2005. The mycorrhizal fungus *Glomus intraradices* and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of *Acacia holosericea*. Soil Biol. Biochem. 37, 1460–1468.

Estaun, V., Save, R., Biel, C., 1997. AM inoculation as a biological tool to improve plant re-vegetation of a disturbed soil with *Rosmarinus officinalis* under semi-arid conditions. Appl. Soil Ecol. 6, 223–229.

Fillion, M., St-Arnaud, M., Fortin, J.A., 1999. Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere micro-organisms. New Phytol. 141, 525–533.

Franson, R.I., Bethlenfalvay, G.J., 1989. Infection unit method of vesicular-arbuscular mycorrhizal propagule determination. Soil Sci. Soc. Am. J. 53, 754–756.

Frey-Klett, P., Chavatte, M., Clausse, M.L., Courrier, S., Le Roux, C., Raajmakers, J., Martinotti, M.G., Pierrat, J.C., Garbaye, J., 2005. Ectomycorrhizal symbiosis affects functional diversity of rhizosphere fluorescent pseudomonads. New Phytol. 165, 317–328.

George, E., Haussler, K., Vetterlein, G., Gorgus, E., Marschner, H., 1992. Water and nutrient translocation by hyphae of *Glomus mosseae*. Can. J. Bot. 70, 2130–2137.

Graham, J.H., Leonard, R.T., Menge, J.A., 1981. Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol. 68, 548–552.

Guissou, T., Bâ, A.M., Guinko, S., Plenchette, C., Duponnois, R., 2001. Mobilisation des phosphates naturels de Kodjari par des jujubiers (*Ziziphus mauritiana* Lam.) mycorhizés dans un sol acidifié avec de la tourbe. Fruits 56, 261–269.

Habte, M., Manjunath, A., 1991. Categories of vesicular-arbuscular mycorrhizal dependency of host species. Mycorrhiza 1, 3–12.

Hafidi, M., 1996. Enrichissement du compost par addition des phosphates naturels. Thèse d'Etat, Faculté des Sciences Semlalia, Marrakech.

Hayman, D.S., 1983. The physiology of vesicular-arbuscular mycorrhizal symbiosis. Can. J. Bot. 61, 944–962.

Heinemeyer, O., Insam, H., Kaiser, E.A., Walenzik, G., 1989. Soil microbial biomass and respiration measurements: an automated technique based on infrared gas analysis. Plant Soil 116, 77–81.

Herrera, M.A., Salamanca, C.P., Barea, J.M., 1993. Inoculation of woody legumes with selected arbuscular mycorrhizal fungi and rhizobia to recover desertified Mediterranean ecosystems. Appl. Environ. Microb. 59, 129–133.

Illmer, P., Schinner, F., 1992. Solubilization of inorganic phosphate by microorganisms isolated from forest soil. Soil Biol. Biochem. 24, 389–395.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum*. I. Spread of hyphae and phosphorus inflow into roots. New Phytol. 120, 371–380.

Jasper, D.A., Abbot, L.K., Robson, A.D., 1989. Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol. 112, 93–99.

Jasper, D.A., Abbot, L.K., Robson, A.D., 1991. The effect of soil disturbance on vesicular-arbuscular mycorrhizal fungi in soils from different vegetation types. New Phytol. 118, 471–476.

John, M.K., 1970. Colorimetric determination of phosphorus in soil and plant material with ascorbic acid. Soil Sci. 68, 171–177.

Landeweert, R., Hoffland, E., Finlay, R.D., Kuyper, T.W., van Breemen, N., 2001. Linking plants to rocks: ectomycorrhizal fungi mobilize nutrients from minerals. Trends Ecol. Evol. 16, 248–253.

Lendzemo, V.W., Kuyper, T.W., Kropff, M.J., van Ast, A., 2005. Field inoculation with arbuscular mycorrhizal fungi reduces *Striga hermonthica* performance on cereal crops and has the plastic bagential to contribute to integrated *Striga* management. Field Crop Res. 91, 51–61.

Linderman, R.G., 1988. Mycorrhizal interactions with the rhizosphere microflora: the mycorrhizosphere effect. Phytopathology 78, 366–371.

Magurran, A.E., 1988. Ecological Diversity and Its Measurement. Croom Helm, London.

Mansfeld-Giese, K., Larsen, J., Bodker, L., 2002. Bacterial populations associated with mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices*. FEMS Microbiol. Ecol. 41, 133–140.

Marshner, P., Baumann, K., 2003. Changes in bacterial community structure induced by mycorrhizal colonization in split-root maize. Plant Soil 251, 279–289.

Marshner, P., Crowley, D.E., 1996. Physiological activity of a bioluminescent *Pseudomonas fluorescens* (strain 2-79) in the rhizosphere of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L.). Soil Biol. Biochem. 28, 869–876.

Marshner, P., Timonen, S., 2005. Interactions between plant species and mycorrhizal colonization on the bacterial community composition in the rhizosphere. Appl. Soil Ecol. 28, 23–36.

Marshner, P., Crowley, D.E., Higashi, M., 1997. Root exudation and physiological status of a root-colonizing fluorescent pseudomonad in mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum* L.). Plant Soil 189, 11–20.

McGee, P., 1989. Variation in propagule numbers of vesicular-arbuscular mycorrhizal fungi in a semi-arid soil. New Phytol. 92, 28–33.

Michelsen, A., Lisanework, N., Friis, I., 1993. Impacts of tree plantations in the Ethiopian highland on soil fertility, shoot and root growth, nutrient utilisation and mycorrhizal colonisation. Forest Ecol. Manage. 61, 299–324.

Mosse, B., 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annu. Rev. Phytopathol. 11, 171–196.

Ouahmane, L., Hafidi, M., Kisa, M., Boumezouch, A., Thioulouse, J., Duponnois, R., 2006. *Lavandula* species as accompanying plants in *Cupressus* replanting strategies: effect on plant growth, mycorrhizal soil infectivity and soil microbial catabolic diversity. Appl. Soil Ecol. 34, 190–199.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161.

Plenchette, C., 2000. Receptiveness of some tropical soils from banana fields in Martinique to the arbuscular fungus *Glomus intraradices*. Appl. Soil Ecol. 15, 253–260.

Plenchette, C., Fortin, J.A., Furlan, V., 1983. Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. I. Mycorrhizal dependency under field conditions. Plant Soil 70, 199–209.

Plenchette, C., Declerck, S., Diop, T., Strullu, D.G., 1996. Infectivity of monoaxenic subcultures of the AM fungus *Glomus versiforme* associated with Ri-TDNA transformed root. Appl. Microbiol. Biotechnol. 46, 545–548.

Posta, K., Marshner, H., Romheld, V., 1994. Manganese reduction in the rhizosphere of mycorrhizal and non-mycorrhizal maize. Mycorrhiza 5, 119–124.

Requena, N., Jeffries, P., Barea, J.M., 1996. Assessment of natural mycorrhizal in a desertified semiarid ecosystem. Appl. Environ. Microb. 62, 842–847.

Requena, N., Perez-Solis, E., Azcon-Aguilar, C., Jeffries, P., Barea, J.M., 2001. Management of indigenous plant-microbe symbioses aids restoration of desertified ecosystems. Appl. Environ. Microb. 67, 495–498.

Ryan, P.R., Delhaise, E., Jones, D.L., 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. 52, 527–560.

Schreiner, R.P., Mihara, K.L., Mc Daniel, H., Benthlenfalvay, G.J., 2003. Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188, 199–209.

Shachar-Hill, Y., Pfeffer, P.E., Douds, D., Osman, S.F., Doner, L.W., Ratcliffe, R.G., 1995. Partitioning of intermediary carbon metabolism in vesicular-arbuscular mycorrhizal leeks. Plant Physiol. 108, 7–15.

Sieverding, E., 1991. Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ), Eschborn, Germany.

Smith, S.E., Read, D.J., 1997. Mycorrhizal Symbiosis, 2nd ed. Academic Press, UK.

Thioulouse, J., Chessel, D., Dolédec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis and graphical display software. Stat. Comp. 7, 75–83.

Timonen, S., Jorgensen, K.S., Haahtela, K., Sen, R., 1998. Bacterial community structure at defined locations of *Pinus sylvestris–Suillus bovinus* and *Pinus sylvestris–Paxillus involutus* mycorrhizospheres in dry pine forest humus and nursery peat. Can. J. Microbiol. 44, 499–513.

Tobar, R.M., Azcon, R., Barea, J.M., 1994. The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhizae. Mycorrhiza 4, 105–108.

van der Hejden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity ecosystem variability and productivity. Nature 396, 69–72.

Vazquez, M.M., Cesar, S., Azcon, R., Barea, J.M., 2000. Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, Trichoderma) and their effects on microbial population and enzymen activities in the rhizosphere of maize plants. Appl. Soil Ecol. 15, 261–272.

West, A.W., Sparling, G.P., 1986. Modifications of the substrate-induced respiration method to permit measurements of microbial biomass in soils of differing water contents. J. Microbiol. Methods 5, 177–189.